REMARKS

Claims 1-23 are pending. Claims 1-6 and 21-23 are withdrawn from consideration as being drawn to a non-elected invention. Claims 21-23 have been canceled. Claims 1, 3-6, 8-10, 12, 13, 15, and 17 have been amended, and claims 24-27 have been added. Support for these amendments is found at paragraphs 0016, 0045, 0049, 0050, 0077-0080 and 0146. Claims 1-20 and 24-27 remain the case.

The examiner has maintained the restriction requirement. As noted in the response to restriction requirement, the MPEP states that, "[i]f the search and examination of the entire application can be made *without serious burden*, [then] the examiner *must* examine on the merits, even though it includes claims to independent or distinct inventions." MPEP §803 (8th ed.) (emphasis added). In response to applicants' election of the claims of Group VII, the examiner searched and examined the claims of Groups I-VI. Thus, the examiner already has shouldered the burden of searching and examining the claims of Groups I-VII. To require applicants now to cancel claims 1-6 would be a waste of both applicants' and the PTO's resources. Reconsideration and withdrawal of the restriction with respect to Groups I-VII is respectfully requested.

Inasmuch as applicants again urge the propriety of the rejoinder of Groups I-VI with Group VII, applicants first will address the rejections of claims 1-6 that were stated in the Official Action dated November 5, 2002, which was superceded by the current Action, which addresses the patentability of Claims 7-20.

Claims 1 and 4-6 were rejected under Section 112, second paragraph. The word "substantially" has been deleted from claim 1. Claim 4 has been amended to depend from claim 1. Support for 56,000 daltons in claim 5 is found in paragraph 0056. Claim 6 has been amended to recite a modified polypeptide according to claim 1 that comprises polyethylene glycol.

Claims 1-6 were rejected under Section 101 and Section 112, first paragraph, because they did not recite SEQ ID NO:10, the complete HAL sequence, and that the specification taught that only SEQ ID NO:10 has enzyme activity. The specification does

not teach that only SEQ ID NO:10 has enzyme activity or even that enzyme activity is required for use in the present context. Indeed, the specification discloses in paragraph 0049 that "within the present invention, moreover, are molecules that do not contain the active site." Clearly enzyme activity is not a prerequisite for the therapeutic activity presently disclosed.

In order to advance prosecution, however, claim 1 has been amended to recite an isolated and purified polypeptide displaying a histidine ammonia lyase activity that is not decreased in the presence of L-histidinol or a therapeutic salt thereof. Also, the recited polypeptide corresponds in sequence to histidine ammonia lyase of *Corynebacteriaceae* or to a fragment thereof that includes the active site, and it may comprise conservative substitutions relative to the *Corynebacteriaceae* histidine ammonia lyase sequence. Numerous examples of fragments that encompass the active site are given, such as SEQ ID Nos: 1-5. Reconsideration of the earlier-stated rejections under Sections 101 and 112 is solicited.

An additional basis of rejection under Section 112, first paragraph, alleged that the claims should recite histidinol instead of histidine analog. This has been done. Furthermore, the recitation in claims 1-6 of the histidine ammonia lyase of *Corynebacteriaceae* or a fragment thereof comprising the active site should obviate this ground of rejection.

Original claims 1-5 were rejected under Section 102(b) based on either Brand *et al.* or Roberts *et al.* Claim 6 is rejected under Section 103(a) based on Brand *et al.* or Roberts *et al.* in view of Shittigar or Kinstler *et al.* Neither of the two primary references discloses or suggests an isolated and purified polypeptide having histidine ammonia lyase activity that corresponds in sequence to histidine ammonia lyase of *Corynebacteriaceae* or to a fragment thereof which includes the active site, as presently claimed. Brand *et al.* discloses a histidase from rat liver. One prevalent characteristic of all known isolated histidine ammonia lyases is their inhibition in the presence of a histidine analog, like histidinol

(specification at paragraph 0008). Accordingly, Brand *et al.* does not disclose a histidase as presently claimed.

While Roberts *et al.* describes a histidase from *Corynebacteriaceae*, as noted in paragraph 0004, Roberts *et al.* only partially characterized the enzyme. More particularly, in Roberts *et al.* cells were suspended in buffer, sonically disrupted, and the sonicate clarified by centrifugation. Partial purification was attained by ammonium sulfate precipitation followed by chromatography on DEAE-Sephadex column. The specific activity of the partially purified L-histidase was only 2.1 IU/mg of protein. As noted in paragraph 0004, "the *Corynebacteriaceae* HAL which Roberts *et al.* described was not in purified form." In order to sequence HAL, a partially purified preparation as disclosed in Roberts *et al.* was resolved by SDS-PAGE, electrophoretically transferred it to Immobilon-P, and stained with Coomassie Brilliant Blue. The major band of 55,000 daltons was excised, and subjected to N-terminal sequencing. In other words, it was purified, and claim 1 has been amended to so indicate. As disclosed in paragraph 0146], purified HAL has been determined to have approximately 40 I.U./mg of activity at 37°C, although the absolute value of activity will vary somewhat depending of various factors. For example, the specification shows in Figure 8 a variation in activity based on pH on HAL activity. The absolute value of activity will also vary with the particular assay used, but the activity of purified HAL clearly is at least an order of magnitude greater than that disclosed in Roberts *et al.*

When HAL is made recombinantly in accordance with one embodiment of the invention, a simple purification method involving two acetone precipitation steps and one Q-sepharose column is used. Following resuspension of the cell paste in one-tenth volume of 50 mM TRIS pH 8.0 the pellet is sonicated four times and centrifuged. An equal volume of acetone is added to the supernatant. The solution is then centrifuged at 14,000 rpm for 15 minutes. The supernatant is retained and an equal volume of acetone is again added and again centrifuged. Following the second acetone precipitation the pellet is resuspended in 50 mM TRIS pH 8.0. The resuspension is then loaded onto a Q-sepharose column (5 mg

protein/ ml Q-sepharose) in 20 mM TRIS pH 8.0. The column is then washed with 20 mM TRIS pH 8.0 with 0.1 M KCl. Elution is performed with a 200 ml gradient from 0.1 M to 0.6 M KCl at a flow rate of 1 ml/min. Phenyl sepharose can then be used to further purify the enzyme. Purification via this scheme is disclosed in Example 6 and depicted in Figure 6. This scheme also leads to purified polypeptide that hence has higher activity than the crude prep disclosed in Roberts *et al.*

Several potential alternate methods of purification also are described in the specification that produce polypeptide with high activity. Because HAL is resistant to heating at 70°C, heating and centrifugation can be used to remove precipitated contaminant proteins. Additionally, HAL does not precipitate with the addition of ammonium sulfate to 30% saturation. Therefore, addition of 30% ammonium sulfate and centrifugation can also be used to remove contaminant proteins. This precipitation can then be followed by purification via a phenyl sepharose column.

Neither Brand *et al.* nor Roberts *et al.* discloses an isolated and purified polypeptide as presently recited in claims 1-6. Shittigar and Kinstler *et al.* are relied upon only as teaching the addition of polyethylene glycol to different enzymes for various reasons, and do not overcome the failings of the primary references. Reconsideration of the earlier-stated rejections under Sections 102 and 103 is solicited.

Turning now to the current Action that is directed to claims 7-20, there are a number of objections that are raised. A proposed drawing correction is appended, for the examiner's consideration and approval. The description of Figure 10 in paragraph 0036 has been corrected. Paragraph 0040, the description of Figure 14, is correct as it stands. The examiner will note that in addition to the 21 numbered sequences of Figure 14, there is an unnumbered sequence against which is each of the 21 sequences is being compared. Thus, there are 22 sequences in Figure 14. Paragraph 0127 has been returned to its original form and Table 1 in paragraph 0128 has been corrected.

Claims 10, 12, and 13 are rejected under the second paragraph of Section 112, and have been amended to eliminate confusing and/or unclear language identified by the examiner.

Claims 7-20 stand rejected under the first paragraph of Section 112 as containing subject matter which was not described in the specification in such a way as to reasonably convey that applicants had possession of the claimed invention. The claims have been amended to recite an isolated polypeptide having about 40 IU/mg protein of histidine ammonia lyase activity, wherein the histidine ammonia lyase activity is not decreased in the presence of L-histidinol or a therapeutic salt thereof and the isolated polypeptide corresponds in sequence to histidine ammonia lyase of *Corynebacteriaceae* or to a fragment thereof which includes the active site, wherein the isolated polypeptide may comprise conservative substitutions relative to the sequence of histidine ammonia lyase of *Corynebacteriaceae*. These amendments address the examiner's concerns that histidine ammonia lyases from organisms other than *Corynebacteriaceae* and histidine analogs other than L-histidinol are not fairly taught.

A further concern expressed by the examiner is that "HAL or any other histidine ammonia lyase [has not] been shown to deliver an immunsuppressant to a patient as in claims 16-20." However, the products of the enzymatic action of histidine ammonia lyase are trans-urocanic acid (t-UA) and ammonia. Irradiation at approximately 310 nm causes the photoisomerization of t-UA to its cis isomer (c-UA). This is disclosed by Hanson *et al.*, *Proc. Natl. Acad. Sci. USA* 95: 10576-10578 (1998). Cis-urocanic acid is taught to play the role of one of the UVB-induced immunosuppressive mediators (Kripke, *Cancer Res.* 54: 6102-6105 (1994); and Norval *et al.*, *Photochem. Photobiol.* 62: 209-217 (1995)). This immunosuppressive property of urocanic acid can be used, for example, to treat immune system disorders and to prevent rejection of transplanted organs.

In a preferred embodiment according to the invention, PEGylated HAL is used. PEGylated HAL has a long circulatory half-life in mice (over 48 hours). Thus, an effective dose (1 μg to 1 g per kg body weight) of a histidine ammonia lyase can be used to generate circulating urocanic acid for prolonged periods of time. This overcomes a problem with

administering urocanic acid which, being a small molecule, is rapidly cleared from circulation. Following administration of HAL, a cis-isomerizing agent such as UVB irradiation is used to cause local immunosuppression (for conditions such as psoriasis), or systemic immunosuppression, the process of which subjects the patient to whole body irradiation. In one example, whole body irradiation can be employed according to the invention, to combat organ rejection following transplantation. In another embodiment, selective immunosuppression can be achieved by targeting the UVB irradiation. For example, psoriasis could be treated by an injection of a histidine ammonia lyase followed by selective irradiation of the affected areas. Selective UVB irradiation, following the injection of a histidine ammonia lyase into a patient, can also be used to treat conditions like arthritis. A skilled artisan would find the factual underpinnings of the method of claims 16-20 to be sound, and thus would not need an actual exemplification in order to make and/or use the subject matter claimed, or to accept that applicants were in possession of this aspect of the invention.

Reconsideration and withdrawal of all bases for rejection under the first paragraph of Section 112 is respectfully requested.

Claims 13 and 14 are rejected under Section 103(a) based on Roberts *et al.* or Jack *et al.* and claim 15 is rejected based on the same two references taken in view of Shittigar or Kinstler *et al.* Claim 13 has been amended to recite a method or reducing toxicity to normal cells from chemotherapeutic agents or retroviral vectors. As described in paragraphs 0076 to 0080, the method of claims 13-15 uses histidine ammonia lyase to deplete circulating histidine. This causes growth arrest in normal cells, without affecting the growth of tumor cells. Chemotherapeutic drugs function preferentially against proliferating cells and are less inclined to react with quiescent cells, so this protects the normal cells from the toxic effects of the chemotherapeutic agent and increases the therapeutic index of cancer chemotherapy.

In yet another aspect of the invention of claims 13-15, a histidine ammonia lyase can be used to enhance the specificity of cancer gene therapy. Retroviral vectors are one of the commonly used vehicles to deliver therapeutic genes for selectively killing tumor cells.

Retroviruses deliver DNA into growing cells without significant capacity to discriminate between cycling normal and cycling tumor tissues. Accordingly, retroviral therapy also suffers from the problem of killing high concentrations of non-targeted, healthy (*i.e.* non-tumor) cells that are proliferating at a given time in the human body. To obviate these problems, a histidine ammonia lyase can be administered to a patient first, thereby causing normal (*i.e.* non-tumor) cells to enter a reversible quiescent state. This treatment would arrest the growth of normal cells without affecting the growth of cancer cells. As a result, retroviral vectors would selectively target proliferating tumor cells.

Based on the foregoing amendments and remarks, all claims are believed to be in condition for allowance, and applicants solicit a prompt notice of the same. Should there be any matter that requires further attention, the examiner is asked to contact the undersigned at the local telephone exchange provided below.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE – SPECIFICATION

Please amend paragraph 0036 as follows:

Figure 10 depicts the effectiveness of L-histidinol as a single agent ~~and in combination with HAL~~. Lane 1: Control. Lane 2: L-histidinol (0.1 mM). Lane 3: L-histidinol (0.5 mM). Lane 4: L-histidinol (1.0 mM). Lane 5: L-histidinol (1.5 mM). Lane 6: L-histidinol 3.0 mM).

Please amend paragraph 0127 as follows:

~~**Two of the resulting probes (TM63 and TM74), shown in Table 1, below, were labeled, mixed, and used to screen the above genomic library. Oligos were labeled with γ^{32}PATP using T4 polynucleotide kinase as described (Ausubel, *et al*, eds, 1994. "Current Protocols in Molecular Biology," John Wiley and Sons, Inc.,) and cleaned up using Elutips (Schleicher & Schuell). Hybridization of duplicate filters was carried out in a Belleo hybridization oven at 37°C using the SSPE protocol as described (Ausubel, *et al.*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994). Filters were washed in 6X SSC with 0.5%SDS (Ausubel, *et al*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994) at 37°C. Filters were then washed at successively higher temperatures in 3 M TMAC (Ausubel, *et al*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994) until very little radioactivity could be detected with a survey meter (generally 45 - 55°C). Upon exposure to X-Ray film (Kodak X-Omat), colonies which were evident on both replicate filters were picked with a wooden toothpick and transferred to a fresh nylon filter overlaid onto an LB/ampicillin plate. This procedure was repeated until a homogeneous population was achieved.~~

~~**Table 1:** oligonucleotides (SEQ ID NOS 1-27, respectively, in order of appearance) with DNA sequence and approximate coordinates relative to the ATG start codon.~~

~~Name~~	~~Length~~	~~Sequence (5' to 3')~~	~~Coordinates~~
~~TM63~~	~~30~~	~~CGCGTTCAGGACGCATACTCCGTTCGCTGC~~	~~838-867~~

~~TM74 24 GCCCATGGAAACGTGGTCTTCCTG 1370-1393~~

~~TM85 21 ATCATCATGCCCGAGTCCACA 1156-1176~~

~~TM87 21 GCCATCAGGAAGACCACGTTT 990-971~~

~~TM89 20 ATGCAGGAAGACCACGTTTC 1246-1265~~

~~TM91 21 ATCGAGGTCCGCCAATGCCAT 648-628~~

~~TM92 18 ACCGGAGCAGCCCAGTGA 441-424~~

~~TM93 20 TGCTTGAAGTATTGCGCCAG 1403-1422~~

~~TM94 18 GATCCTCGGGTGCGATGT 226-209~~

~~TM95 18 ATGCTGATCGGGCTTCGT 92-74~~

~~TM96 27 ATTTGATTCATATGGCTTCCGCTCCTC -11 +16~~

~~TM97 28 ATCTTGGATCCGAACATGGTGCGTTGCA Beyond C-Terminus~~

~~TM98 18 AGCACCAGAT CGATGCAC 128-145~~

~~TM99 18 TGGCATGGGTGAACCGGT 267-284~~

~~TM101 18 ATCAGCGTTGAAGCCCAG 682-699~~

~~TM103 18 ACGTGCTGGACTTCCTTG 1019-1036~~

~~TM105 18 GTGCATAAGGCCCTCGAA 1501-1518~~

~~TM106 18 GAGCTTCGAGGGCCTTAT 1522-1505~~

~~TM109 18 CGAGCAACGCAGCGAGTA 870-853~~

DNA probes were designed using regions of known histidine ammonia lyases that had a strong probability of being conserved in HAL. Using the Wisconsin Graphics GCG

package pileup program, the peptide sequences of the known histidine ammonia lyases from *B. subtilis*, *S. griseus*, *P. putida*, and rat were aligned and examined for highly conserved regions. Several of these were chosen as candidates for probe design. Using the DNA sequences of cloned genes from *Corynebacterium* , a codon preference table was derived. From this a backtranslation was performed resulting in the most likely DNA sequence for the protein region of interest.

Please amend paragraph 0128 as follows:

Two of the resulting probes (TM63 and TM74), shown in Table 1, below, were labeled, mixed, and used to screen the above genomic library. Oligos were labeled with γ^{32}PATP using T4 polynucleotide kinase as described (Ausubel, *et al*, eds, 1994. "Current Protocols in Molecular Biology," John Wiley and Sons, Inc.,) and cleaned up using Elutips (Schleicher & Schuell). Hybridization of duplicate filters was carried out in a Bellco hybridization oven at 37°C using the SSPE protocol as described (Ausubel, *et al.*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994). Filters were washed in 6X SSC with 0.5%SDS (Ausubel, *et al*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994) at 37°C. Filters were then washed at successively higher temperatures in 3 M TMAC (Ausubel, *et al*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994) until very little radioactivity could be detected with a survey meter (generally 45 - 55°C). Upon exposure to X-Ray film (Kodak X-Omat), colonies which were evident on both replicate filters were picked with a wooden toothpick and transferred to a fresh nylon filter overlaid onto an LB/ampicillin plate. This procedure was repeated until a homogeneous population was achieved.

Table 1: oligonucleotides (SEQ ID NOS 13-31, respectively, in order of appearance) with DNA sequence and approximate coordinates relative to the ATG start codon.

Name	Length	Sequence (5' to 3')	Coordinates
TM63	30	CGCGTTCAGGACGCATACTCCGTTCGCTGC	838-867

TM74	24	GCCCATGGAAACGTGGTCTTCCTG	1370-1393
TM85	21	ATCATCATGCCCGAGTCCACA	1156-1176
TM87	21	GCCATCAGGAAGACCACGTTT	990-971
TM89	20	ATGCAGGAAGACCACGTTTC	1246-1265
TM91	21	ATCGAGGTCCGCCAATGCCAT	648-628
TM92	18	ACCGGAGCAGCCCAGTGA	441-424
TM93	20	TGCTTGAAGTATTGCGCCAG	1403-1422
TM94	18	GATCCTCGGGTGCGATGT	226-209
TM95	18	ATGCTGATCGGGCTTCGT	92-74
TM96	27	ATTTGATTCATATGGCTTCCGCTCCTC	-11- +16
TM97	28	ATCTTGGATCCGAACATGGTGCGTTGCA	Beyond C-Terminus
TM98	18	AGCACCAGAT CGATGCAC	128-145
TM99	18	TGGCATGGGTGAACCGGT	267-284
TM101	18	ATCAGCGTTGAAGCCCAG	682-699
TM103	18	ACGTGCTGGACTTCCTTG	1019-1036
TM105	18	GTGCATAAGGCCCTCGAA	1501-1518
TM106	18	GAGCTTCGAGGGCCTTAT	1522-1505
TM109	18	CGAGCAACGCAGCGAGTA	870-853

Please amend paragraph 0133 as follows:

HAL from a bacterium from the family *Corynebacteriaceae* that had been partially purified using ammonium sulfate and DEAE - Sephadex was resolved by SDS-PAGE. The separated material was electrophoretically transferred to Immobilon-P and stained with Coomassie Brilliant Blue. The major band of 55 000 daltons was excised and subjected to N-terminal sequencing. This fraction was sent to Commonwealth Biotechnologies, Inc. (Richmond, VA), cleaved with BrCN, HPLC purified, and fractions sequenced. ~~The data are shown in Figure 2.~~

VERSION WITH MARKINGS TO SHOW CHANGES MADE - CLAIMS

1. (Amended) An isolated and purified polypeptide having histidine ammonia lyase activity, wherein [said] the histidine ammonia lyase activity is not [substantially] decreased in the presence of [a histidine analog] L-histidinol or a therapeutic salt thereof and the isolated polypeptide corresponds in sequence to histidine ammonia lyase of *Corynebacteriaceae* or to a fragment thereof that includes the active site, wherein the isolated polypeptide may comprise conservative substitutions relative to the sequence of histidine ammonia lyase of *Corynebacteriaceae*.

3. (Amended) [An isolated] A polypeptide according to claim 1, comprising a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6.

4. (Amended) [An isolated] A polypeptide according to claim [3] 1, wherein the polypeptide has a monomeric molecular weight between about 30,000 [to] and 70,000 daltons

5. (Amended) [An isolated] A polypeptide according to claim 4, wherein the polypeptide has a monomeric molecular weight of about 56,000 daltons.

6. (Amended) A [method for PEGylating a polypeptide, comprising reacting a PEG with the polypeptide with the] modified polypeptide according to claim 1, that comprises polyethylene glycol.

8. (Amended) A method of treatment according to claim 7, wherein the histidine ammonia lyase activity is not [substantially] decreased in the presence of [a histidine analog] L-histidinol or a therapeutic salt thereof and the polypeptide corresponds in sequence to histidine ammonia lyase of *Corynebacteriaceae* or to a fragment thereof which includes the active site, wherein the polypeptide may comprise conservative substitutions relative to the sequence of histidine ammonia lyase of *Corynebacteriaceae*.

9. (Amended) A method according to claim 8, wherein the [histidine analog is histidinol] histidine ammonia lyase activity is not decreased in the presence of L-histidinol.

10. (Amended) A method according to claim 8, further comprising administering [to a patient in need of treatment] a therapeutic amount of [a histidine analog] L-histidinol or a therapeutic salt thereof.

12. (Amended) A method for treating a patient suffering from a cancer, comprising administering to the patient suffering from said cancer a therapeutic amount of [the polypeptide in claim 1] a polypeptide having histidine ammonia lyase activity, wherein said histidine ammonia lyase activity is not decreased in the presence of L-histidinol or a therapeutic salt thereof and the polypeptide corresponds in sequence to histidine ammonia lyase of *Corynebacteriaceae* or to a fragment thereof which includes the active site, wherein the polypeptide may comprise conservative substitutions relative to the sequence of histidine ammonia lyase of *Corynebacteriaceae*, and a therapeutic amount of [a histidine analog] L-histidinol or a therapeutic salt thereof.

13. (Amended) A method for [treating disease] reducing toxicity to normal cells from chemotherapeutic agents or retroviral vectors, comprising

(i) administering to a patient a therapeutically effective amount of a polypeptide having histidine ammonia lyase activity, and

(ii) additionally administering to said patient a therapeutically effective amount of a chemotherapeutic agent or retroviral vector, whereby said polypeptide having histidine ammonia lyase activity selectively depletes circulating histidine and causes growth arrest in normal cells, without affecting the growth of tumor cells.

15. (Amended) A method according to claim 13, wherein the polypeptide is a [PEGylated] modified polypeptide that comprises polyethylene glycol.

17. (Amended) A method according to claim 16, wherein the irradiating agent is UVB irradiation, and wherein the polypeptide [is a PEGylated polypeptide] comprises polyethylene glycol.